

Sidell, J. S. Urbanetti, W. J. Smith, and C. G. Hurst in *Textbook of Military Medicine, Medical Aspects of Chemical and Biological Warfare*, edited by F. R. Sidell, E. T. Takafuji, and D. R. Franz (Office of the Surgeon General at TMM Publications, Washington, D. C. 1997) p 201. The LD<sub>50</sub> for soman (GD) is 350 mg/70 kg man. See F. R. Sidell in *Textbook of Military Medicine, Medical Aspects of Chemical and Biological Warfare*, edited by F. R. Sidell, E. T. Takafuji, and D. R. Franz (Office of the Surgeon General at TMM Publications, Washington, D. C. 1997) p 141. These two values allow us to rank the active TSP formulations and to select the appropriate component for advanced development.

**[0054]** The proof-of-neutralization test is used to verify that active TSP formulations actually neutralize CWAs into less toxic materials. This test uses the headspace solid phase microextraction (HS-SPME) technique for the collection of CWAs. Samples collected on the extraction filament are analyzed by gas chromatography/mass spectroscopy. 100 mg of active TSP formulation are challenged with 0.1  $\mu$ l of neat CWA (HD, GD, or VX) in a small vial. The headspace above the mixture is sampled periodically to determine the amount of CWA remaining in the flask. Efficacy is determined by the % loss of CWA. Other analytical techniques such as Nuclear Magnetic Resonance (NMR) and Fourier-Transform Infrared Spectrometry (FTIR) have also been used in this module.

**[0055]** Formulations that pass this initial set of screens are moved into the second phase of testing using animal models. The weakling pig test for HD vapor evaluates a 0.15 mm thick layer of active TSP spread on the depilated dorsa. The standard saturated vapor cup is used for a 30 min challenge. The effectiveness of the active TSP is determined by measuring the degree of erythema that developed on the skin exposure site. Erythema is measured objectively using a reflectance calorimeter (see Braue, E. H. Jr. *Journal of Applied Toxicology*, 1999, 19(S), S47-S53).

layer of active TSP spread on the clipped dorsa is challenged with 1.0  $\mu$ l of liquid HD spread by a 12 mm disk. The effectiveness of the active TSP is determined by measuring the lesion areas of protected and non-protected sites.

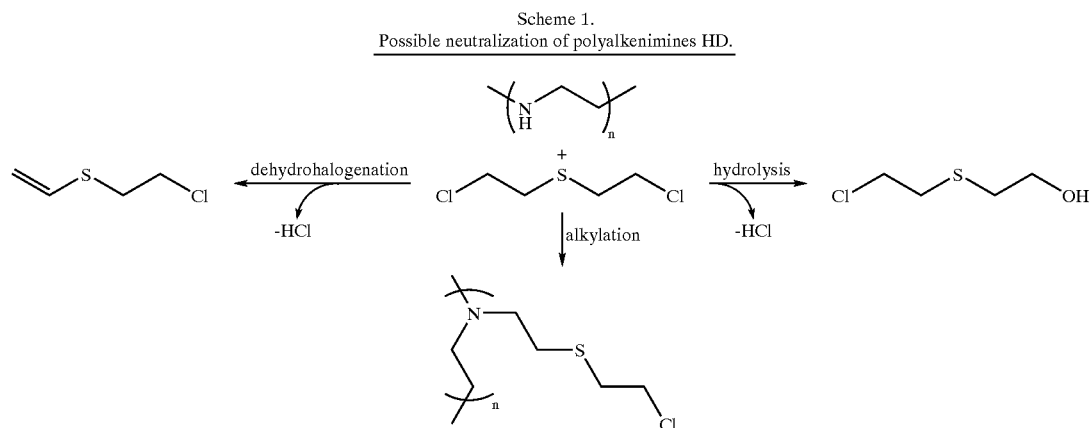
**[0057]** The rabbit lethality test is used to evaluate a challenge by GD vapor. In this test, a 0.15 mm layer of active TSP spread on the clipped dorsa is challenged with two vapor cups (7 cm<sup>2</sup> each) containing 28 mg GD per kg of body weight. The liquid GD is saturated onto filter paper fitted into the top of the cap so that liquid can not run down. The caps are left in place for 4 hours. After the exposure period the caps are removed and the exposure sites decontaminated. This exposure dose is lethal to all animals not protected with active TSP. The effectiveness of the active TSP is determined by lethality 1, 2, 3, 4 and 24 hr following exposure.

**[0058]** The guinea pig lethality test is performed by applying a 0.15 mm thick layer of active TSP on the clipped dorsa of guinea pig followed by a fixed dose of GD, TGD or VX. The effectiveness of the active TSP is determined by lethality 1, 2, 3, 4 and 24 hr following exposure.

**[0059]** Candidate formulations that pass the in vivo test modules move into advanced animal testing. These tests are similar to the initial animal tests with the addition of stresses for wear-time and washing with a simulated sweat solution. Interactions with other products that a warfighter might use are also evaluated. These products include camouflage paints, sunscreens and insecticides.

#### **[0060] Results**

**[0061]** The polyalkenimines are effective active moieties reducing the amount of HD vapor by >99% relative to the TSP alone. Although the exact mechanisms for HD neutralization are not clear, they may react by hydrolysis, direct alkylation of the polyalkenimine, or dehydrohalogenation (Scheme 1).



**[0056]** The rabbit lesion area ratio (LAR) test is used to evaluate a challenge by HD liquid. In this test, a 0.15 mm

**[0062]** Against GD vapor the polyalkenimines reduced the amount of GD by >99% compared to TSP alone. In this case,